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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Bernauer et al. Group: 126

Serial No. 07/686,210, filed April 16, 1991

Examiner: Reamer, J.

For: **CATECHOL DERIVATIVES**

DECLARATION OF GERHARD ZÜRCHER UNDER RULE 132

Basle, Switzerland
December 15, 1992

Honorable Commissioner of Patents & Trademarks
Washington, D.C. 20231

I, Gerhard Zürcher declare the following:

I am a citizen of Switzerland, residing at 116 Grenzacherstrasse, CH-4058 Basle, Switzerland,

I was awarded a degree as a chemical engineer from the Burgdorf Institute of Technology, in 1974,

From 1974 up to the present, I have been employed in the field of neuropharmacological research by F.Hoffmann-La Roche Ltd, Basle, Switzerland. Throughout that time, I have been evaluating compounds for neuropharmacological properties in in vitro and in vivo testing.

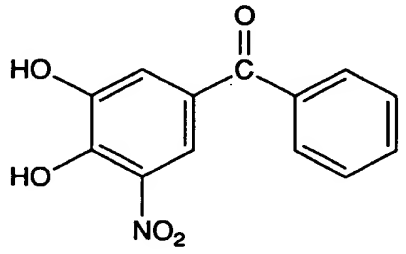
I am a co-inventor of the subject matter of U.S. Patent Application Serial No. 07/686,210, filed April 16, 1991,

I am familiar with the prior art cited in connection with the prosecution of the captioned application, that is, Kitahara, et al. U.S. Patent No. 4,622,066, issued November 11, 1986; GB 967605; Miyamoto et al. Chemical Abstracts, Vol. 99, 121998 (1983); and Watsuka EP 79141; October 1982.

Compound B is specifically described in Watsuka, that is, in reference example 4, pages 27-28 of the Watsuka reference.

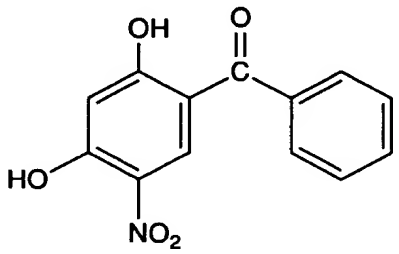
The testing described below were performed at my direction and under my supervision to determine the activity of the following compounds in inhibiting catechol-O-methyltransferase.

Compound of the Invention



Compound A

Compound of the Prior Art



Compound B

The methodology employed and the results obtained when compounds of the invention and of the prior art were tested for their activity in inhibiting catechol-O-methyltransferase are described hereinafter.

METHODOLOGY

Catechol-O-methyltransferase (COMT) activity *in vitro* was measured by a rapid radiochemical assay. By this procedure, the substrate catechol is converted to tritiated guaiacol by incubating rat liver homogenates at 37°C for 15 minutes in the presence of (^{3}H)methyl-S-adenosyl-1-methionine, Mg^{2+} and adenosine deaminase at pH 7.6 See, G. Zürcher and M. Da Prada, Rapid and sensitive single-step radiochemical assay for catechol-O-methyltransferase, J. Neurochem. 38: 191-195, 1982 a copy of which is attached. The test compounds, dissolved in 25 µl dimethylsulfoxide were added to the incubation cocktail in the following (final) concentrations in triplicate:

Compound A: 10, 20, 50, 100, 200, 500 and 1000 nmoles/1

Compound B: 1, 3, 10, 30, 100, 300, 1000, 3000 and 10000 μmoles/1

At the end of the incubation period, ^{3}H-guaiacol formed enzymatically was extracted into a mixture of hexane and toluene (5:1) and counted in a β-counter. The concentration of the compound leading to a 50% inhibition of the enzymatic activity (IC_{50}) was calculated from the computer-fitted sigmoid dose response curve.

RESULTS

TABLE I

Compound A	$IC_{50} = 4.8 \times 10^{-8}$ moles/l
Compound B	$IC_{50} = 4.4 \times 10^{-3}$ moles/l

DISCUSSION

As can be seen from the data, Compound A achieves a 50% inhibition of catechol-O-methyl transferase activity at a substantially lower concentration than Compound B. In other words, it would take 100,000 times more of Compound B, the compound of the prior art, than of Compound A to achieve the same benefit. Based on my experience, compounds having an IC_{50} greater than 10^{-7} moles/l do not have the potency to inhibit the COMT in vivo at reasonable doses.

METHODOLOGY

The activity in vivo was measured as follows: Test compounds, suspended in water (containing 1% Tween 80) were administered orally to groups of 4 male albino rats (160-180 g

body weight) at a dose of 100 mg/kg. Six untreated rats served as the control group. One hour later, the rats were decapitated, the brains, livers and kidneys taken out and frozen immediately in dry ice. Livers and kidneys were homogenized in 50 and brains in 10 volumes of ice-cold water containing 0.2% Triton X-100 and 0.002% dithiothreitol and then centrifuged at 35'000 x g for 20 minutes at 4°C. In a manner similar to the *in vitro* test, 50 µl (liver, kidney) or 100 µl (brain) of the supernatant were incubated in the presence of catechol, (^{3}H)methyl-S-adenosyl-L-methionine, Mg^{2+} and adenosine deaminase at pH 7.6. After incubating for 15 minutes (liver, kidney) or 30 minutes (brain) at 37°C ^{3}H-guaiacol was extracted into hexane/toluene 5:1 and counted in a β-counter. The results, provided in Table II below, are a measure of the percentage of enzymatic activity present after administration of the test compounds compared to untreated animals.

RESULTS

TABLE II

	% of Control Group		
	Brain	Liver	Kidney
Compound A	23.1 ± 0.7	11.8 ± 0.7	4.5 ± 0.3
Compound B	86.5 ± 5.0	91.6 ± 4.1	91.7 ± 5.0

Means ± SEM

DISCUSSION

As can be seen from the foregoing data, the administration of equivalent amounts of Compound A and Compound B in vivo, results in significantly greater inhibition of catechol-O-methyl transferase activity upon administration of Compound A as compared to Compound B.

CONCLUSION

When comparing the results set forth in Tables I and II, it is clear that, representative Compound A of the invention has greater COMT inhibitory activity than Compound B of the prior art which renders the compounds of the invention more useful.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that all statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Gerhard Zürcher

Dated: December 15, 1992

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